High-dose Vitamin A With Vaccination After 6 Months of Age: A Randomized Trial

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KEY WORDS

vitamin A supplementation, child mortality, vaccinations, randomized controlled trial

ABBREVIATIONS

BCG—Bacille Calmette-Guérin vaccine
DTP—diphtheria tetanus pertussis vaccine
DSMB—data safety and monitoring board
MV—measles vaccine
MRR—mortality rate ratio
OPV—oral polio vaccine
VAS—vitamin A supplementation
WHO—World Health Organization
YF—yellow fever vaccine

Dr Fisker designed the trial, designed the data collection instruments, supervised data collection, data entry and data cleaning, analyzed the data, drafted the initial manuscript, and revised and approved the final manuscript; Drs Bale and Rodrigues supervised data collection and data entry, critically reviewed the manuscript, and approved the final manuscript as submitted; Mr Balde and Mr Fernandes supervised data collection and data entry, reviewed the manuscript, and approved the final manuscript as submitted; Dr Jørgensen supervised data collection, analyzed the blood samples, critically reviewed the manuscript, and approved the final manuscript as submitted; Mr Danneskiold-Samsøe analyzed the blood samples, critically reviewed the manuscript, and approved the final manuscript as submitted; Drs Hornshøj, Rasmussen, and Christensen supervised data collection and data entry, critically reviewed the manuscript, and approved the final manuscript as submitted; Dr Bibby provided input and statistical support for data analyses, reviewed and critically revised the manuscript, and approved the final manuscript as submitted; Dr Aaby designed the trial, designed and reviewed the data collection instruments, provided input to data analyses, reviewed and critically revised the manuscript, and approved the final manuscript as submitted; and Dr Benn conceptualized and designed the trial, designed and reviewed the data collection instruments, provided input to data analyses, critically reviewed the manuscript, and approved the final manuscript as submitted.

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WHAT'S KNOWN ON THIS SUBJECT: The World Health Organization recommends using vaccination contacts to deliver high-dose vitamin A supplementation (VAS) to children aged 6 to 59 months. The effect of this policy on overall child mortality has not been assessed.

WHAT THIS STUDY ADDS: In this first randomized controlled trial of VAS at routine vaccination contacts after 6 months, VAS had no overall effect on mortality but was associated with reduced mortality in girls and increased mortality in boys.

abstract

BACKGROUND: The World Health Organization recommends vitamin A supplementation (VAS) at routine vaccination contacts after 6 months of age based on the assumption that it reduces mortality by 24%. The policy has never been evaluated in randomized controlled trials for its effect on overall mortality. We conducted a randomized double-blind trial to evaluate the effect of VAS with vaccines.

METHODS: We randomized children aged 6 to 23 months 1:1 to VAS (100 000 IU if aged 6–11 months, 200 000 IU if aged 12–23 months) or placebo at vaccination contacts in Guinea-Bissau. Mortality rates were compared in Cox proportional-hazards models overall, and by gender and vaccine.

RESULTS: Between August 2007 and November 2010, 7587 children were enrolled. Within 6 months of follow-up 80 nonaccident deaths occurred (VAS: 38; placebo: 42). The mortality rate ratio (MRR) comparing VAS versus placebo recipients was 0.91 (95% confidence interval 0.59–1.41) and differed significantly between boys (MRR 1.92 [0.98–3.75]) and girls (MRR 0.45 [0.24–0.87]) (P = .003 for interaction between VAS and gender). At enrollment, 42% (3161/7587) received live measles vaccine, 29% (2154/7587) received inactivated diphtheria-tetanus-pertussis–containing vaccines, and 21% (1610/7587) received both live and inactivated vaccines. The effect of VAS did not differ by vaccine group.

CONCLUSIONS: This is the first randomized controlled trial to assess the effect of the policy on overall mortality. VAS had no overall effect, but the effect differed significantly by gender. More trials to ensure an optimal evidence-based vitamin A policy are warranted. *Pediatrics* 2014;134:e739–e748

Vitamin A supplementation (VAS) to children older than 6 months is estimated to reduce all-cause mortality by 24%[1] based primarily on randomized trials conducted in the 1980s and early 1990s, before the vaccination program had reached high coverage. The beneficial effect of VAS is ascribed to prevention of vitamin A deficiency.[2–4] However, this explanation fits poorly with available data: the effect does not depend on the degree of deficiency,[5] no beneficial effect is seen in deficient children aged 1 to 5 months,[6,7] and smaller doses may be better than larger doses.[7,8] We have hypothesized that the effect of VAS is modified by vaccines, VAS amplifying the nonspecific immune-modulating effects of vaccines, thus being beneficial when provided with live vaccines but potentially harmful with inactivated vaccines.[9] This would explain why VAS is not beneficial for children aged 1 to 5 months who typically receive inactivated diphtheria-tetanus-pertussis (DTP) vaccine, but beneficial later when children receive live measles vaccine (MV). Supporting this hypothesis, in a randomized trial to study the effects of VAS with MV on antibody response, we found that VAS with MV was associated with a mortality rate ratio (MRR) of 0.46 (0.14–1.47).[10] In an observational study of VAS provided with vaccines in a campaign, the effect of VAS with DTP was significantly worse than the effect of VAS with MV.[11]

The World Health Organization (WHO) recommends VAS at routine vaccination contacts after 6 months of age.[12,13] Roughly 200 million children in the 103 priority countries receive VAS every year, often with vaccines.[14,15] The effect of combining VAS with vaccines on overall mortality has never been evaluated in randomized trials. According to the vaccination program in most low-income countries, the first vaccine after 6 months of age is the live MV recommended at 9 months of age. However, vaccinations are often delayed and many children receive an inactivated DTP or pentavalent (DTP combined with Hepatitis B and *Haemophilus influenzae* type B) vaccine after 6 months of age.[16] If the effect of VAS is modified by vaccines, the effect of providing VAS with vaccines as compared with VAS alone could be very different.

In Guinea-Bissau, VAS is provided in campaigns, not at routine vaccination contacts. We conducted an individually randomized and double-blinded trial to test the effect on overall mortality of the WHO policy of providing VAS with vaccines. Based on previous studies,[10,11] we hypothesized that VAS with MV would be associated with reduced mortality, but VAS with DTP vaccine would be associated with increased mortality. Previous studies have indicated that effects of VAS differ by gender;[8,17–21] we had therefore prespecified stratified analyses by gender. Season and previous VAS have been important determinates of the effect of VAS,[18,22,23] and stratified analyses by these parameters were also prespecified.

METHODS

Setting and Population

The trial was conducted in urban and rural areas surveyed through the Health and Demographic Surveillance System of the Bandim Health Project (www.bandim.org) in Guinea-Bissau (Supplemental Information). Guinea-Bissau has 2 distinct seasons: a dry season (December–May) and a rainy season (June–November).[18] WHO estimates that 55% (10%– 93%) of preschool children in Guinea-Bissau are vitamin A deficient.[24] During the year before initiation of the trial, mortality in the rural areas surveyed by the Bandim Health Project was 23.8/1000 person-years of observation (PYRS) among children aged 6 to 59 months (AB Fisker et al, unpublished data).

When we initiated the trial in August 2007, the recommended vaccination schedule was Bacille Calmette-Guérin (BCG) vaccine and oral polio vaccine (OPV) at birth; DTP with OPV at 6, 10, and 14 weeks; and MV at 9 months of age. In August 2008 the schedule was altered: pentavalent vaccines replaced DTP and yellow fever (YF) vaccine was administered with MV at 9 months of age.

Identification of Eligible Children, Informed Consent, and Baseline Examination

Enrollment into the trial took place between August 13, 2007, and December 28, 2009, in the urban area and between September 11, 2007, and November 28, 2010, in the rural area. Children due to be vaccinated were invited to participate in the trial at health centers and during outreach in the urban study area and at vaccination posts set up in the villages during 6-monthly visits in the rural area. Exclusion criteria were VAS within the preceding month and taking part in another trial. Enrollment was initially limited to children 6–17 months but was extended to 23 months in the rural area from February 2008 because there were many older children missing vaccinations.

After the consent procedure (Supplemental Information), children were weighed, and length and mid-upper-arm-circumference were measured. Finger prick blood samples were obtained and analyzed from 15% of the participants to assess vitamin A status[26] (Supplemental Information).

Randomization and Masking

Children were randomized 1:1 to VAS or placebo in blocks of 20 stratified by gender. Children aged 6 to 11 months

received 0.5 mL vitamin A (100 000 IU) or 0.5 mL placebo oil, and children aged 12 to 23 months received 1 mL vitamin A (200 000 IU) or 1 mL placebo oil. Children received vitamin A or placebo oils only at the time of randomization.

Samples from all 5 batches of VAS and placebo were tested for vitamin A content at "AS Vitas," Oslo, Norway. The first batch contained only 69% of the 200 000 IU/mL when tested 8 months after production in April 2008. This batch was immediately replaced. The lowest concentration for the other 4 batches was 94% when tested 4, 14, 22, and 28 months after production.

Vaccines were UNICEF certified and delivered through the national vaccination program (Supplemental Information).

Outcomes

The main outcome was mortality. Secondary outcomes were morbidity, growth, vitamin A status, and immunologic effects. As specified in the protocol, secondary outcomes are reported elsewhere.[26,27] The follow-up for mortality ended at whichever came first: subsequent VAS, 12 months of follow-up, first visit after February 24, 2011 (when another trial was initiated), or at latest July 21, 2011 when the code was broken. When a death was registered, an interview was conducted on the circumstances leading to the death. Follow-up of children who died due to accidents was censored on the date of death (Supplemental Table 6).

VAS Campaigns

When the trial was planned, Guinea-Bissau had annual VAS campaigns. However, the national policy changed, and biannual campaigns providing VAS to all children aged 6 to 59 months took place during the full duration of the trial.

Sample Size

No previous trial has examined the effect of VAS with vaccines after 6 months of age on mortality. Sample size calculations were based on the hypotheses that VAS with MV would reduce mortality by 40%, whereas VAS with DTP vaccine would be associated with 30% increased mortality until reception of subsequent VAS or a different vaccine. We had anticipated an annual mortality of 8% to 9% in the younger DTP recipients and 5% in the slightly older MV recipients, and 50% censoring of follow-up due to other vaccines, migration, and VAS campaigns among DTP recipients and 10% among MV recipients. During the conduct of the trial, blinded interim analyses prepared for the Data Safety and Monitoring Board (DSMB) revealed that mortality was considerably lower than anticipated (2% and 1%) and that we therefore would be unable to achieve conclusive results with the planned sample size of 6000. The sample size was consequently increased to 9500, which was logistically feasible with continued rural enrollments for 2 years. A stopping rule of $P = .025$ in either gender was defined by the DSMB. After a planned interim analysis in November 2010, which demonstrated conclusive evidence of benefit or harm in girls, enrollments were stopped when 7587 children were enrolled. Results are reported for the entire cohort; the effect of VAS was similar when the analysis was restricted to the first 6000 children and when 1927 children receiving the first lower-potency batch were excluded (Supplemental Table 7).

Statistical Analyses

Survival was assessed in Cox proportional-hazards models with age as underlying time. Analyses were performed using Stata 11.2 (Stata Corp, College Station, TX).

The unforeseen increased frequency of VAS campaigns posed analytical challenges. Information on campaign VAS was obtained during home visits and thus available only for surviving children. Using the information obtained during visits could introduce bias, and censoring all children at the date of a campaign severely reduced the follow-up time and compromised the power. Conducting follow-up to 12 months of age would mean that most children had received VAS twice during follow-up and this would lead to dilution of any effect of the intervention. Hence, we conducted 2 types of analyses. In the main analysis, we followed children for 6 months after enrollment, irrespective of subsequent VAS; this main analysis includes some follow-up time after campaign VAS which may have diluted the effect of the experimental treatment. In a restricted analysis, we censored follow-up time at the date of the next VAS campaign in the community. This analysis censors too much time, as not all children receive VAS in the campaigns. We also conducted an analysis with follow-up to 12 months.

In the analyses stratified by type of vaccine, we defined MV as MV given with or without YF, and DTP vaccine as either DTP vaccine or pentavalent vaccine. Combined live and inactivated vaccinations were defined as MV ($\pm$YF) and DTP/pentavalent vaccines administered simultaneously.

As prespecified, all analyses considered interaction between VAS and gender[8,17–21] and, in addition, previous VAS[22,23] and season.[18] Interactions between VAS and the suspected modifier were tested by using Wald test statistics.

Ethical Considerations

The protocol was approved by the Ministry of Health in Guinea-Bissau and the Danish Central Ethical Committee gave its consultative approval (2006-7041-99).

RESULTS

We enrolled 7587 children (VAS: 3787, placebo: 3800). The background factors were comparable between the 2 randomization groups (Table 1). Two-thirds of the 1102 blood samples indicated vitamin A deficiency. Vitamin A status did not differ by gender (Table 1).[26] Eight deaths due to accidents (VAS: 2, placebo: 6) (Supplemental Table 6) were censored on the date of death (Fig 1), leaving 80 nonaccident deaths (VAS: 38, placebo: 42) within 6 months and 137 nonaccident deaths (VAS: 66; placebo: 71) within 12 months of follow-up.

Mortality for VAS Versus Placebo

In the main analysis with 6 months of follow-up, there was no overall effect of VAS (MRR = 0.91 [0.59–1.41]) but the effect differed significantly for boys (MRR = 1.92 [0.98–3.75]) and girls (MRR = 0.45 [0.24–0.87]) (P = .003 for interaction between VAS and gender) (Table 2, Fig 2). Including the deaths due to accidents, the MRR was 0.87 (0.57–1.34), 1.56 (0.83–2.93) for boys and 0.49 (0.26–0.92) for girls (P = .01 for interaction between VAS and gender). No single main symptom preceding death explained the differential effect of VAS in the 2 genders (Supplemental Information).

In the restricted analysis censoring follow-up at the date of the first VAS campaign after enrollment, there were 36 nonaccident deaths (18 VAS, 18 placebo); the median follow-up time was 2.9 months (interquartile range: 1.9–4.4). VAS had no effect on survival, the MRR being 1.00 (0.52–1.93) (Table 2); but the effect differed by gender, being 2.31 (0.89–6.01) for boys and 0.34 (0.11–1.05) for girls (P = .01 for interaction between VAS and gender).

With 12 months of follow-up, the estimates were 0.93 (0.67–1.30) overall, 1.22 (0.77–1.95) for boys and 0.69 (0.43–1.13) for girls (P = .10 for interaction between VAS and gender) (Table 2).

Mortality by Vaccine at Enrollment

Among both VAS and placebo recipients, the mortality rate was lowest among children who received MV alone (Supplemental Information). A total of 6925 (92%) children were randomized to VAS or placebo with MV (+/−YF) only (42%), DTP/pentavalent vaccine only (29%) or a combination of these vaccines (21%) (Table 1). The 614 children receiving other combinations of vaccines were not included in this analysis (Table 3). At least one-third of the children in the DTP/pentavalent only group received MV during follow-up, whereas few children in the MV group received additional vaccines during follow-up (Supplemental Table 8). VAS was not significantly associated with survival in any of the vaccine groups (Table 3). In all vaccine groups the effect tended to be negative for boys and beneficial for girls. The gender-differential effect of VAS was strongest in the group receiving both MV and DTP, being statistically significant in both the main (Table 3) and restricted analyses as well as with the full 12 months of follow-up (data not shown).

Stratified Analyses by Season and Previous VAS

In the main analysis, the effect of VAS was not significantly different in the dry (MRR=0.79 (0.43–1.45)) and the rainy season (MRR=1.06 (0.56–2.02)). Neither gender benefited from VAS in the rainy season but a strong gender-differential effect was observed in the rainy season (P = .005) (Table 4). No single main symptom preceding death explained the strong gender-differential effect of VAS in the dry season (Supplemental Information).

More than half of the children had received VAS previously (Table 1). The effect of VAS differed by previous VAS: VAS was strongly beneficial in girls who had previously received VAS, the MRR being 0.18 (0.05–0.62) but less so in girls who had not (MRR = 0.82 [0.35–1.89]) (P = .05 for interaction between VAS and previous VAS). The effect in previously supplemented boys was negative (MRR = 5.98 [1.34–26.7]), whereas there was no strong effect in boys who had not received VAS on a previous occasion (MRR = 1.19 [0.53–2.65]), P = .06 for a differential effect in boys). The opposite effects in boys and girls were reflected in a 3-way interaction among VAS, gender, and previous VAS (P = .007) (Table 5).

DISCUSSION

This is the first randomized placebo-controlled trial to test the effect on mortality of the current WHO policy of providing VAS at routine vaccination contacts after 6 months of age, an intervention that is assumed to reduce mortality by 24%.[1] In spite of the high prevalence of vitamin A deficiency, VAS with vaccines did not lower overall mortality but had strong gender-differential effects, VAS being associated with a reduction in female mortality but an increase in male mortality. We found little support for our hypothesis that the effect of VAS would differ by whether it was coadministered with MV or DTP, but the trial confirmed a recent finding, that previous VAS primes a positive response to subsequent doses in girls.[22]

Strengths and Weaknesses

The use of the Health and Demographic Surveillance System platform minimized loss to follow-up. However, the power of the trial was limited because of a lower than anticipated mortality and the increased frequency of national VAS campaigns shortening the follow-up period. We increased power by extending the trial. We obtained similar

TABLE 1 The Distribution of Baseline Characteristics Between the Vitamin A and the Placebo Groups

	All		Boys		Girls	
	Vitamin A	Placebo	Vitamin A	Placebo	Vitamin A	Placebo
No.	3760	3779	1899	1915	1861	1864
Age, mo, median (interquartile range)	9.8 (8.7–12.5)	9.8 (8.8–12.6)	9.7 (8.5–12.3)	9.9 (8.9–12.5)	9.9 (8.9–12.6)	9.8 (8.8–12.6)
Old EPI vaccines[a]	1615 (43)	1612 (43)	809 (43)	799 (42)	806 (43)	813 (44)
OPV	225 (14)	177 (11)	123 (15)	86 (11)	102 (13)	91 (11)
MV	660 (41)	678 (42)	337 (41)	341 (43)	323 (40)	337 (41)
DTP	388 (24)	400 (25)	192 (24)	196 (25)	196 (24)	204 (25)
DTP+MV	342 (21)	357 (22)	157 (19)	176 (22)	185 (23)	181 (22)
New EPI vaccines[a]	2088 (56)	2120 (56)	1058 (56)	1096 (57)	1030 (55)	1024 (55)
YF	93 (4)	78 (4)	41 (4)	43 (4)	52 (5)	35 (3)
MV+YF	887 (42)	936 (44)	459 (43)	490 (45)	428 (42)	446 (44)
Pentavalent	694 (33)	672 (32)	362 (34)	348 (32)	332 (32)	324 (32)
Pentavalent+MV	83 (4)	78 (4)	32 (3)	42 (4)	51 (5)	36 (4)
Pentavalent+YF	13 (1)	10 (0)	7 (1)	3 (0)	6 (1)	7 (1)
Pentavalent+MV+YF	318 (15)	346 (16)	157 (15)	170 (15)	161 (16)	176 (17)
Combination of old and new EPI vaccines[a]	57 (2)	47 (1)	32 (2)	20 (1)	25 (1)	27 (1)
DTP+YF	9 (16)	9 (19)	4 (13)	3 (15)	5 (20)	6 (22)
DTP+MV+YF	48 (84)	38 (81)	28 (88)	17 (85)	20 (80)	21 (78)
Received VAS before enrollment[b]	2026 (54)	2060 (55)	1014 (53)	1017 (53)	1020 (55)	1049 (56)
Enrolled in rural area[b]	2288 (61)	2299 (61)	1134 (60)	1145 (60)	1154 (62)	1154 (62)
Enrolled in the dry season[b]	1707 (45)	1712 (45)	851 (45)	860 (45)	856 (46)	852 (46)
Anthropometrics at enrollment						
Mean weight-for-age (SD)[c]	−0.89 (1.23)	−0.85 (1.23)	−0.93 (1.28)	−0.92 (1.27)	−0.85 (1.17)	−0.78 (1.19)
Mean length-for-age (SD)[c]	−0.94 (1.37)	−0.94 (1.48)	−0.99 (1.44)	−1.07 (1.50)	−0.89 (1.29)	−0.80 (1.44)
Mean weight-for-length (SD)[c]	−0.50 (1.26)	−0.45 (1.24)	−0.52 (1.34)	−0.46 (1.30)	−0.48 (1.17)	−0.44 (1.18)
Mean arm-circumference-for-age (SD)[c]	−0.18 (1.12)	−0.17 (1.13)	−0.19 (1.16)	−0.19 (1.16)	−0.17 (1.07)	−0.14 (1.09)
Mean maternal arm-circumference, mm (SD)	271 (33)	272 (33)	271 (33)	271 (33)	270 (33)	272 (33)
Breastfed at enrollment[b,d]	3689 (98)	3686 (98)	1869 (99)	1871 (98)	1820 (98)	1815 (98)
Morbidity on day of enrollment[b,d]						
Diarrhea	325 (9)	370 (10)	162 (9)	185 (10)	163 (9)	185 (10)
Cough	855 (23)	817 (22)	449 (24)	421 (22)	406 (22)	396 (21)
Fever	567 (15)	561 (15)	297 (16)	288 (15)	270 (15)	273 (15)
Vomiting	168 (4)	186 (5)	100 (5)	85 (4)	68 (4)	101 (5)
Socioeconomic status						
Formal education of mother[b,d]	1635 (46)	1620 (45)	834 (46)	815 (45)	801 (45)	805 (46)
Mother signed enrollment form	1265 (34)	1253 (33)	658 (35)	642 (34)	607 (33)	611 (33)
Ethnicity[b,d]						
Balanta	782 (21)	769 (21)	377 (20)	370 (19)	405 (22)	399 (21)
Fula	675 (18)	698 (19)	352 (19)	356 (19)	323 (17)	342 (18)
Mandinga	505 (14)	469 (13)	253 (13)	240 (13)	252 (14)	229 (12)
Pepel	792 (21)	810 (22)	402 (21)	420 (22)	390 (21)	390 (21)
Manjaco/Mancanha	432 (12)	423 (11)	232 (12)	208 (11)	200 (11)	215 (12)
Other	553 (15)	582 (16)	273 (14)	307 (16)	280 (15)	275 (15)
Age of mother, y, median (interquartile range)	26 (22–31)	26 (22–31)	26 (22–31)	26 (22–31)	26 (22–31)	26 (21–31)
Vitamin A deficient[e]	374 (67)	350 (64)	192 (68)	180 (66)	182 (67)	170 (62)

Values are numbers (percentages) unless stated otherwise. EPI, Expanded Program on Immunizations.

[a] Vaccine groups: OPV may have been given together with the vaccines.

[b] Variables in 2 levels are presented by 1 level.

[c] Z-scores for weight-for-age, length-for-age, weight-for-length, and arm-circumference-for-age were based on the 2006-WHO child growth standard.

[d] Values do not add up because some have missing information.

[e] Vitamin A status assessed among 1102 participants, VAS: 557, Placebo: 545.

effect estimates when the analysis was limited to the first 6000 enrolled children, when follow-up was censored at the next VAS campaign (restricted analysis), as well as when the children were followed for 6 or 12 months, indicating robust results and similar conclusions also in a setting with biannual VAS campaigns.

The change in the vaccination program leading to many different vaccine groups limited our power to describe effects of VAS by vaccination status. Other vaccines received after enrollment but before the next home visit during which the vaccines were registered are likely to have modified the observed effect. Furthermore, having received VAS previously appeared to have a major impact on the response to subsequent doses;

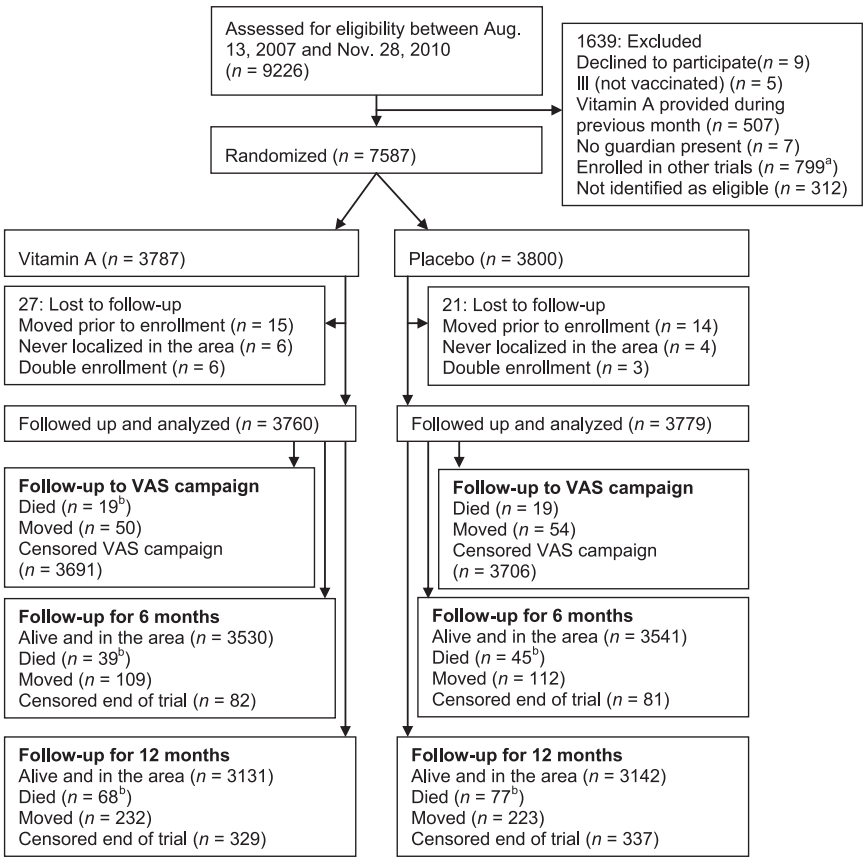

FIGURE 1

Trial diagram. [a]Enrolled in other trials (all urban area): randomized controlled trial of VAS with BCG in normal birth weight infants (n = 494), randomized controlled trials of BCG in low birth weight infants (n = 246), and randomized controlled trial of not giving DTP with or after MV (n = 59). [b]Deaths due to accidents described in Supplemental Table 6.

this observation[22] had not yet been made when the study was planned. Hence, the results for specific types of vaccines should be interpreted with caution; larger studies are needed to resolve when and with which vaccines VAS has a beneficial effect.

TABLE 2 The Effect of VAS at Vaccination Contacts on All-Cause Mortality, Overall and by Gender

	Rate per 1000 PYRS# (Deaths/PYRS)		MRR (95% Confidence Interval)[a]	Test of Interaction Between VAS and Gender[b]
	Vitamin A	Placebo		
Follow-up for 6 mo				
All	20.6 (38/1845)	22.7 (42/1849)	0.91 (0.59–1.41)	
Boys	26.8 (25/932)	13.9 (13/936)	1.92 (0.98–3.75)	0.003
Girls	14.2 (13/913)	31.8 (29/913)	0.45 (0.24–0.87)	
Follow-up to next VAS campaign				
All	19.0 (18/945)	19.0 (18/948)	1.00 (0.52–1.93)	
Boys	29.2 (14/479)	12.5 (6/478)	2.31 (0.89–6.01)	0.01
Girls	8.6 (4/466)	25.6 (12/469)	0.34 (0.11–1.05)	
Follow-up for 12 mo				
All	18.8 (66/3516)	20.1 (71/3526)	0.93 (0.67–1.30)	
Boys	21.9 (39/1777)	17.9 (32/1787)	1.22 (0.77–1.95)	0.10
Girls	15.5 (27/1739)	22.4 (39/1739)	0.69 (0.43–1.13)	

#, person-years of observation.

[a] Analyzed by using Cox proportional-hazards models with age as underlying time scale.

[b] Tested using Wald test.

The Effect of VAS on Overall Mortality

As recognized by WHO, no randomized study has assessed the impact of co-administration of VAS and vaccines after 6 months on child mortality.[13] VAS provided independently of vaccines to children aged 6 to 59 months was studied extensively in the 1980s and early 1990s. A recent meta-analysis based on these trials estimated that VAS reduces overall mortality by 24%.[1] However, there are several indications also from the original trials that VAS is not always associated with reductions in mortality. Among the original 8 trials,[28] 2 did not find beneficial effects.[29,30] In a reanalysis of one of the trials that reported a beneficial effect,[31] we found no effect among vaccinated children.[32] In more recent studies, we observed negative effects of VAS when provided to neonate girls who subsequently received DTP[33,34] and when provided to children who had received BCG revaccination.[35] The original trials documenting beneficial effects before the full implementation of the vaccination program[1] may therefore not reflect the effect of VAS today in the context of much higher vaccination coverage and repeated doses of VAS. Our trial indicates that VAS may no longer be associated with strong benefits. Consistent with this possibility, a cluster randomized open trial of 6-monthly VAS found no effect among 1 million Indian children.[36] If the effect of VAS on mortality has changed over time, this is obviously extremely important.

Interpretation

The effect of VAS is ascribed to the prevention and treatment of vitamin A deficiency.[2–4] However, there is accumulating evidence that vitamin A deficiency alone does not define whether VAS will be beneficial.[9] Several observations in the present trial support that

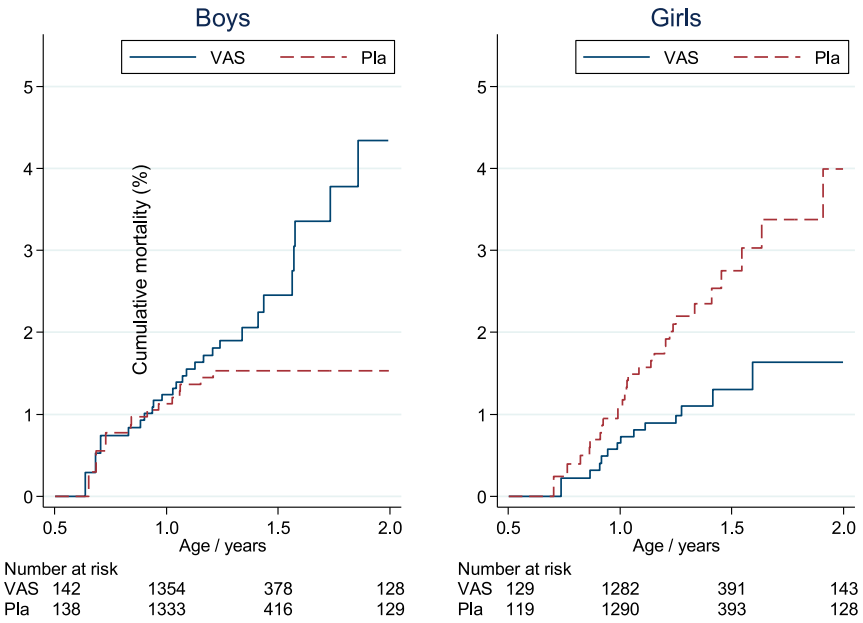

FIGURE 2

Cumulative mortality according to gender and randomization to VAS/placebo. Note: Follow-up censored after 6 months of follow-up.

the effect of VAS cannot be predicted based on vitamin A status. First, vitamin A deficiency was common, but VAS had no overall effect on mortality. Second, vitamin A status was similar in boys and girls but the effect of VAS differed significantly between the genders. Third, the effect of VAS was if anything better in the dry season when vitamin A status is better.[26,37] Fourth, if deficiency was essential, VAS should be most beneficial among children who have not received VAS previously. That was not the case. In contrast, we confirmed a previous finding that girls benefited more from VAS if they had received previous VAS. Therefore, although VAS alleviates vitamin A deficiency, the effect of VAS on the immune system must be taken into account in planning the VAS program. Otherwise, negative effects may counterbalance the benefits obtained from treating vitamin A deficiency.

TABLE 3 The Effect of VAS by Vaccine at Enrollment on All-Cause Mortality, Overall and by Gender

	Rate per 1000 PYRS# (Deaths/PYRS)		MRR (95% Confidence Interval)[a]	Test of Interaction Between VAS and Gender[b]
	Vitamin A	Placebo		
Live				
MV/MV+YF	*n* = 1547	*n* = 1614		
All	13.2 (10/760)	10.1 (8/791)	1.29 (0.51–3.28)	
Boys	17.9 (7/391)	7.4 (3/405)	2.40 (0.62–9.29)	0.18
Girls	8.1 (3/368)	13.0 (5/386)	0.62 (0.15–2.61)	
Inactivated				
DTP or pentavalent	*n* = 1082	*n* = 1072		
All	28.3 (15/530)	26.6 (14/527)	1.06 (0.51–2.20)	
Boys	33.0 (9/273)	15.0 (4/267)	2.16 (0.67–7.03)	0.11
Girls	23.3 (6/257)	38.5 (10/260)	0.61 (0.22–1.69)	
Live and inactivated				
MV/MV+YF + DTP/Pentavalent	*n* = 791	*n* = 819		
All	20.6 (8/389)	40.1 (16/399)	0.52 (0.22–1.21)	
Boys	32.9 (6/182)	20.2 (4/198)	1.66 (0.47–5.89)	0.02
Girls	9.7 (2/206)	59.9 (12/200)	0.16 (0.04–0.73)	

Excluding 402 children who received OPV only (4 deaths; 3 VAS: 2 boys + 1 girl, 1 Placebo: boy), 171 children receiving YF only (3 deaths; All placebo: 1 boy + 2 girls), 18 children receiving DTP + YF (2 deaths, both VAS: boys + girls), 23 children receiving pentavalent + YF (0 deaths); follow-up for 6 months. #, person-years of observation.

[a] Analyzed by using Cox proportional-hazards models with age as underlying time scale.

[b] Tested by using Wald test.

Potential Interactions With Gender and Vaccines

In the original VAS trials, 3 of the 5 trials reporting gender-specific effects found a better effect of VAS in boys than in girls.[38] We had hypothesized that VAS with MV would be beneficial and particularly beneficial for girls. We found the trend for girls but surprisingly we found the opposite trend for boys. In previous studies, we found that neonatal VAS benefited boys but not girls.[18,34] In these studies, children had not received VAS previously and the beneficial effect seen for girls in the present trial may partly be explained by a beneficial effect in girls after repeated doses of VAS.[22,23] We recently observed that neonatal VAS followed by 3 doses of DTP and an early MV was particularly harmful for boys.[39] Thus, the combination of VAS and several vaccines may not benefit boys. In the present trial, we were not able to confirm the observation that VAS provided with DTP was particularly bad for girls, but it should be noted that at least one-third of the DTP-vaccinated children received MV during follow-up.

Although our study concurs with previous studies showing a beneficial effect in girls of repeated doses of vitamin A,[22,23,40] we have not previously observed that a negative effect should be boosted in boys receiving repeated doses. In light of the very low mortality in placebo boys who have previously received VAS, we cannot rule out that it could be a chance finding.

TABLE 4 The Effect of VAS at Vaccination Contacts on All-Cause Mortality During 6 Months of Follow-up by Season of Supplementation

	Rate per 1000 PYRS# (Deaths/PYRS)		Mortality Rate Ratio (95% Confidence Interval)[a]	Test of Interaction Between VAS and Gender[b]
	Vitamin A	Placebo		
Dry season				
	n = 1707	*n* = 1712		
All	22.6 (19/841)	28.6 (24/839)	0.79 (0.43–1.45)[c]	
Boys	33.4 (14/419)	9.5 (4/422)	3.51 (1.16–10.7)[d]	0.0005
Girls	11.8 (5/423)	48.0 (20/417)	0.25 (0.09–0.67)[d]	
Rainy season				
	n = 2053	*n* = 2067		
All	18.9 (19/1004)	17.8 (18/1011)	1.06 (0.56–2.02)[c]	
Boys	21.4 (11/514)	17.5 (9/514)	1.21 (0.50–2.93)[d]	0.67
Girls	16.3 (8/490)	18.1 (9/497)	0.90 (0.35–2.34)[d]	

#, person-years of observation.
[a] Analyzed by using Cox proportional-hazards models with age as underlying time scale.
[b] Tested using Wald test.
[c] $P = .52$ for interaction between VAS and season.
[d] $P = .02$ for interaction among VAS, gender, and season.

TABLE 5 The effect of VAS at Vaccination Contacts on All-Cause Mortality During 6 Months of Follow-up by Previous Reception of Vitamin A

	Rate per 1000 PYRS# (Deaths/PYRS)		MRR (95% Confidence Interval)[a]	Test of Interaction Between VAS and Gender[b]
	Vitamin A	Placebo		
No previous VAS				
	n = 1726	*n* = 1713		
All	27.2 (23/845)	27.5 (23/837)	0.99 (0.57–1.77)	
Boys	29.9 (13/435)	25.0 (11/440)	1.19 (0.53–2.65)[c]	0.53
Girls	24.4 (10/410)	30.2 (12/398)	0.82 (0.35–1.89)[c,d]	
Previous VAS				
	n = 2034	*n* = 2066		
All	15.0 (15/1000)	18.8 (19/1012)	0.80 (0.41–1.58)	
Boys	24.1 (12/497)	4.0 (2/496)	5.98 (1.34–26.7)[c]	0.0004
Girls	6.0 (3/503)	33.0 (17/516)	0.18 (0.05–0.62)[c,d]	

#, person-years of observation.
[a] Analyzed by using Cox proportional-hazards models with age as underlying time scale.
[b] Tested using Wald test.
[c] $P = .007$ for interaction among VAS, gender, and previous VAS.
[d] $P = .05$ for interaction between VAS and previous VAS in girls.

A previous study showed that neonatal VAS was beneficial in the dry but not in the rainy season.[18] In the current study, we observed the same tendency for girls, but not for boys. We have no good explanation for the seasonal difference; in particular, we found no differential pattern in main symptoms preceding death by season of enrollment, which explains the gender-differential effect in the dry season. We speculate that it could be due to VAS amplifying the beneficial nonspecific effects of MV, which are stronger in the dry than in the rainy season.[41] We know of no mechanism that explains the gender-differential effects and urge that the effects of preventive childhood interventions, including VAS and vaccines, are examined by gender. By identifying consistencies and contradictions, we may come closer to an understanding of the biology.

CONCLUSIONS

Currently, VAS is provided at vaccination contacts in 75 countries.[42] The present trial is the first randomized trial to study the overall mortality effect of the policy. In contrast to current assumptions,[1] we found no overall effect of VAS. Given the lack of an overall effect, more randomized trials are warranted to justify the WHO policy. We need to examine whether the beneficial effect of VAS has waned over time and to identify when VAS is beneficial. Importantly, we need to ensure that we do not give VAS when it might be harmful.

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REFERENCES

1. Mayo-Wilson E, Imdad A, Herzer K, Yakoob MY, Bhutta ZA. Vitamin A supplements for preventing mortality, illness, and blindness in children aged under 5: systematic review and meta-analysis. *BMJ*. 2011;343:d5094
2. Humphrey JH, Rice AL. Vitamin A supplementation of young infants. *Lancet*. 2000;356(9227):422–424
3. Humphrey JH, West KP Jr, Sommer A. Vitamin A deficiency and attributable mortality among under-5-year-olds. *Bull World Health Organ*. 1992;70(2):225–232
4. Underwood BA. Vitamin A deficiency disorders: international efforts to control a preventable "pox." *J Nutr*. 2004;134(1):231S–236S

5. Beaton GH, Martorell R, McCabe G, L'abbé KA, Edmonston B, Ross AC. Effectiveness of vitamin A supplementation in the control of young child morbidity and mortality in developing countries— Nutrition policy discussion paper No. 13. Geneva, Switzerland: United Nations Administrative Committee on Coordination/Sub-Committee on Nutrition; 1993
6. West KP Jr, Katz J, Shrestha SR, et al. Mortality of infants < 6 mo of age supplemented with vitamin A: a randomized, double-masked trial in Nepal. *Am J Clin Nutr*. 1995;62(1):143–148
7. WHO/CHD Immunisation-Linked Vitamin A Supplementation Study Group. Randomised trial to assess benefits and safety of vitamin A supplementation linked to immunisation in early infancy. *Lancet*. 1998;352 (9136):1257–1263
8. Benn CS, Martins C, Rodrigues A, Jensen H, Lisse IM, Aaby P. Randomised study of effect of different doses of vitamin A on childhood morbidity and mortality. *BMJ*. 2005;331(7530):1428–1432
9. Benn CS, Balé C, Sommerfelt H, Friis H, Aaby P. Hypothesis: vitamin A supplementation and childhood mortality: amplification of the non-specific effects of vaccines? *Int J Epidemiol*. 2003;32(5):822–828
10. Benn CS, Aaby P, Balé C, et al. Randomised trial of effect of vitamin A supplementation on antibody response to measles vaccine in Guinea-Bissau, west Africa. *Lancet*. 1997; 350(9071):101–105
11. Benn CS, Martins C, Rodrigues A, et al. The effect of vitamin A supplementation administered with missing vaccines during national immunization days in Guinea-Bissau. *Int J Epidemiol*. 2009;38(1):304–311
12. Integration of vitamin A supplementation with immunization. *Wkly Epidemiol Rec*. 1999;74(1):1–6
13. World Health Organization. Guideline: Vitamin A supplementation in infants and children 6–59 months of age. 2011. Available at: www.who.int/nutrition/publications/micronutrients/guidelines/vas_6to59_months/en/index.html. Accessed July 11, 2014
14. UNICEF, World Health Organization. Immunisation summary; a statistical reference containing data through 2009. 2011 edition. Available at: www.childinfo.org/files/32775_UNICEF.pdf. Accessed July 4, 2014
15. UNICEF. Vitamin A supplementation. A decade of progress. 2011. Available at: www.unicef.org/publications/index_39363.html. Accessed July 11, 2014
16. Hornshøj L, Benn CS, Fernandes M, Rodrigues A, Aaby P, Fisker AB. Vaccination coverage and out-of-sequence vaccinations in rural Guinea-Bissau: an observational cohort study. *BMJ Open*. 2012;2(6):e001509
17. Benn CS, Fisker AB, Jørgensen MJ, Aaby P. Why worry: vitamin A with DTP vaccine? *Vaccine*. 2007;25(5):777–779
18. Benn CS, Diness BR, Roth A, et al. Effect of 50,000 IU vitamin A given with BCG vaccine on mortality in infants in Guinea-Bissau: randomised placebo controlled trial. *BMJ*. 2008;336(7658):1416–1420
19. Humphrey JH, Agoestina T, Wu L, et al. Impact of neonatal vitamin A supplementation on infant morbidity and mortality. *J Pediatr*. 1996;128(4):489–496
20. Rahmathullah L, Tielsch JM, Thulasiraj RD, et al. Impact of supplementing newborn infants with vitamin A on early infant mortality: community based randomised trial in southern India. *BMJ*. 2003;327 (7409):254
21. Sommer A, Tarwotjo I, Djunaedi E, et al. Impact of vitamin A supplementation on childhood mortality. A randomised controlled community trial. *Lancet*. 1986;1 (8491):1169–1173
22. Fisker AB, Aaby P, Rodrigues A, Frydenberg M, Bibby BM, Benn CS. Vitamin A supplementation at birth might prime the response to subsequent vitamin A supplements in girls. Three year follow-up of a randomized trial. *PLoS ONE*. 2011;6(8):e23265
23. Yakymenko D, Benn CS, Martins C, et al. The impact of different doses of vitamin A supplementation on male and female mortality. A randomised trial from Guinea-Bissau. *BMC Pediatr*. 2011;11:77
24. World Health Organization. Global prevalence of vitamin A deficiency in populations at risk 1995–2005: WHO global database on vitamin A deficiency. Available at: http://whqlibdoc.who.int/publications/2009/9789241598019_eng.pdf. 2009. Accessed July 11, 2014
25. Fisker AB, Rodrigues A, Martins C, Byberg S, Thysen S, Storgaard L, et al. Reduced child mortality after general measles vaccination campaign in rural Guinea-Bissau (Submitted). 2014
26. Danneskiold-Samsøe N, Fisker AB, Jørgensen MJ, et al. Determinants of vitamin A deficiency in children between 6 months and 2 years of age in Guinea-Bissau. *BMC Public Health*. 2013;13(1):172
27. Fisker AB, Bale C, Jørgensen MJ, et al. High-dose vitamin A supplementation administered with vaccinations after 6 months of age: sex-differential adverse reactions and morbidity. *Vaccine*. 2013;31 (31):3191–3198
28. Fawzi WW, Chalmers TC, Herrera MG, Mosteller F. Vitamin A supplementation and child mortality. A meta-analysis. *JAMA*. 1993;269 (7):898–903
29. Herrera MG, Nestel P, el Amin A, Fawzi WW, Mohamed KA, Weld L. Vitamin A supplementation and child survival. *Lancet*. 1992; 340(8814):267–271
30. Vijayaraghavan K, Radhaiah G, Prakasam BS, Sarma KV, Reddy V. Effect of massive dose vitamin A on morbidity and mortality in Indian children. *Lancet*. 1990;336(8727): 1342–1345
31. Ghana VAST Study Team. Vitamin A supplementation in northern Ghana: effects on clinic attendances, hospital admissions, and child mortality. *Lancet*. 1993;342(8862): 7–12
32. Benn CS, Aaby P, Nielsen J, Binka FN, Ross DA. Does vitamin A supplementation interact with routine vaccinations? An analysis of the Ghana Vitamin A Supplementation Trial. *Am J Clin Nutr*. 2009;90(3): 629–639
33. Benn CS, Rodrigues A, Yazdanbakhsh M, et al. The effect of high-dose vitamin A supplementation administered with BCG vaccine at birth may be modified by subsequent DTP vaccination. *Vaccine*. 2009;27 (21):2891–2898
34. Benn CS, Fisker AB, Napirna BM, et al. Vitamin A supplementation and BCG vaccination at birth in low birthweight neonates: two by two factorial randomised controlled trial. *BMJ*. 2010;340:c1101
35. Roth AE, Benn CS, Ravn H, et al. Effect of revaccination with BCG in early childhood on mortality: randomised trial in Guinea-Bissau. *BMJ*. 2010;340:c671
36. Awasthi S, Peto R, Read S, Clark S, Pande V, Bundy D; DEVTA (Deworming and Enhanced Vitamin A) team. Vitamin A supplementation every 6 months with retinol in 1 million pre-school children in north India: DEVTA, a cluster-randomised trial. *Lancet*. 2013;381 (9876):1469–1477
37. Fisker AB, Lisse IM, Aaby P, et al. Effect of vitamin A supplementation with BCG vaccine at birth on vitamin A status at 6 wk and 4 mo of age. *Am J Clin Nutr*. 2007;86(4):1032–1039
38. Benn CS, Lund S, Fisker A, Jørgensen MJ, Aaby P. Should infant girls receive micronutrient supplements? *Int J Epidemiol*. 2009;38(2):586–590
39. Aaby P, Martins CL, Garly ML, et al. Nonspecific effects of standard measles vaccine at 4.5 and 9 months of age on childhood mortality: randomised controlled trial. *BMJ*. 2010;341:c6495
40. Fisker AB, Aaby P, Bale C, et al. Does the effect of vitamin A supplements depend on vaccination status? An observational study

from Guinea-Bissau. *BMJ Open*. 2012;2(1): e000448

41. Martins CL, Benn CS, Andersen A, et al. A randomized trial of a standard dose of Edmonston-Zagreb measles vaccine given at 4.5 months of age: effect on total hospital admissions. *J Infect Dis*. 2014;209(11): 1731–1738
42. World Health Organization. Immunization service delivery and accelerated disease control. Vitamin A; opportunities to link vitamin A supplementation with immunization. 2013. Available at: www.who.int/immunization/programmes_systems/interventions/vitamin_A/en/index2.html. Accessed July 11, 2014

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